

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/23955>

Please be advised that this information was generated on 2017-12-05 and may be subject to change.



Glomerular filtration and saturable absorption of iohexol in the rat isolated perfused kidney

Rosalinde Masereeuw, Miek M. Moons, Paul Smits & ¹Frans G.M. Russel

Department of Pharmacology, University of Nijmegen, Nijmegen, The Netherlands

1 The renal handling of iohexol was examined in the rat isolated perfused kidney (IPK) over a perfusate concentration range of 5–20 $\mu\text{g ml}^{-1}$.

2 At a concentration of 5 $\mu\text{g ml}^{-1}$, a ratio of renal clearance over clearance by glomerular filtration (Cl_R/GF) of 0.63 ± 0.06 could be determined. This ratio increased until 1.02 ± 0.06 at 20 $\mu\text{g ml}^{-1}$, indicating that a saturable mechanism is involved in the luminal disappearance of the drug.

3 Pretreatment of the kidneys with polylysine, probenecid or diatrizoate resulted in a significantly enhanced clearance of iohexol, probably due to inhibition of membrane binding. Renal clearance data were fitted to a kinetic model including filtration into the primary urine followed by saturable absorption at the luminal membrane. An absorption constant, K_A , of $7.3 \pm 1.3 \mu\text{g ml}^{-1}$, and a maximum rate of absorption, $V_{A,\text{Max}}$, of $1.4 \pm 0.1 \mu\text{g min}^{-1}$ were determined.

4 Iohexol accumulated in kidney tissue, reaching a concentration of 2 to 7.5 times the perfusate concentration. In freshly isolated proximal tubular cells and kidney cortex mitochondria, iohexol reduced the uncoupled respiratory rate at a concentration comparable to the highest tissue concentration found in the IPK.

5 In conclusion, iohexol is not only filtered by the kidney but also reabsorbed via a saturable mechanism, which results in tubular accumulation. Intracellularly sequestered iohexol may affect mitochondrial oxidative metabolism. Our results indicate that iohexol is not a true filtration marker.

Keywords: Perfused kidney; iohexol; contrast agent; glomerular filtration; membrane binding; saturable absorption; tubular accumulation; mitochondrial respiration

Introduction

Iodinated contrast media are widely used for the imaging of organs and blood vessels. The currently used intravascular contrast media are derivatives of triiodinated benzoic acids, and most compounds are excreted primarily by the kidney. While being cleared the contrast agent is concentrated by the kidney and provides good visualization of the entire renal system. All triiodinated contrast media are hyperosmolar, metabolically stable, and can be divided roughly into two major groups; the ionic, high osmolar ($1,200\text{--}2,000 \text{ mOsm l}^{-1}$), and nonionic and relatively low osmolar agents ($300\text{--}650 \text{ mOsm l}^{-1}$) (Morris & Fischer, 1986; Bakris, 1993; Sovak, 1994).

A drawback in the use of radiocontrast media is the risk of acute renal failure. Factors involved in nephropathy are renal haemodynamic alterations, direct tubular cell injury and tubular obstruction (Bakris, 1993; Porter, 1994). Although nephrotoxicity has been shown to be more severe with the use of high-osmolar contrast agents (Thomsen *et al.*, 1988), in patients with pre-existing renal failure the incidence of contrast nephropathy was not significantly different when comparing high and low osmolar media (Barrett *et al.*, 1992). Nonionic contrast media have even been shown to induce more morphological changes in proximal tubules of the kidney. For iohexol, vacuolization was observed in proximal convoluted tubular cells (Tervahartiala *et al.*, 1991; Beaufils *et al.*, 1995). Heyman *et al.* (1988) suggested that the vacuoles are developed by invagination of membranes of lateral cellular interdigitations. However, it has also been speculated that the vacuoles might be secondary lysosomes, in which the contrast medium is sequestered, and formed after the drug enters tubular cells from the tubular lumen via endocytosis (Nordby *et al.*, 1990). If the renal handling of contrast agents comprises endocytosis and, subsequently, accumulation within proximal tubular cells,

high intracellular concentrations may directly affect tubular cell integrity and be the basis of the nephropathy induced. However, the mechanism of direct tubular cell injury remains to be elucidated (Bakris, 1993). Among a variety of suggested mechanisms, some studies have shown that a reduction in renal oxidative metabolism can occur, which may be caused by a diminished oxygen supply or a direct interaction with mitochondrial respiration (Humes *et al.*, 1987; Heyman *et al.*, 1988; Bakris, 1993).

The existence of an endocytotic mechanism is in contradiction with other data indicating that iohexol may be useful as a marker for the determination of glomerular filtration rate (GFR). In these studies the contrast agent was supposed to be handled by the kidney in a fashion similar to inulin, implying that neither secretion nor reabsorption occurred (Frennby *et al.*, 1994; Lindblad & Berg, 1994).

In this study we examined the renal handling of iohexol in the rat isolated perfused kidney (IPK). Previously, we showed that the IPK is a useful model for studying the renal clearance and accumulation of drugs, and their effects on kidney function (Cox *et al.*, 1991; Boom *et al.*, 1994). The purpose of this study was to investigate the presence and role of a reabsorptive mechanism in the overall clearance of iohexol in the IPK, and the effect of intracellularly sequestered drug on mitochondrial oxidative metabolism. The results reveal that iohexol clearance is determined by filtration and saturable absorption, resulting in tubular accumulation. Moreover, the intracellular sequestration of iohexol appears to affect mitochondrial oxidative metabolism.

Methods

Experimental procedure

The isolation and perfusion of the rat kidney has been described in detail previously (Cox *et al.*, 1990). Pluronic F-108 was used as an oncotic agent in the albumin-free perfusion

¹ Author for correspondence at: Department of Pharmacology 233, Faculty of Medical Sciences, University of Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands

fluid. For the determination of glomerular filtration rate (GFR), cyanocobalamin was added to the perfusion fluid. GFR was monitored on line by a micro flow-through cuvette in which the cyanocobalamin concentration was measured colorimetrically (Brink & Slegers, 1979). The experimental period was 120 min and started after a 30 min baseline period. During the baseline period the perfusate volume was 500 ml from which a sample of 5 ml was drawn. After the baseline period, the experimental perfusion fluid was connected to the kidney, with a total volume of 250 ml in which iohexol was already dissolved. Doses added to the perfused kidneys were 0 (time controls), 1.25, 1.88, 2.5 and 5.0 mg of iohexol, resulting in initial perfusate concentrations of 5, 7.5, 10, and 20 $\mu\text{g ml}^{-1}$. Compounds used to affect iohexol clearance were a contrast analogue, diatrizoate, an inhibitor of organic anion transport, probenecid, and an inhibitor of aminoglycoside brush-border membrane binding, polylysine. These agents were added to the IPK at the start of the baseline period, and remained in the perfusion fluid during the experimental period. Urine samples were collected during control and experimental periods over 10 min intervals. Perfusate samples (300 μl) were drawn at the midpoint of each urine collection interval. Two additional perfusate samples were taken, one at the beginning of the experimental period ($t=0$), and one at the end of the experiment. At the end of the experiment the kidney was removed from the system, blotted, weighed, and frozen until analysis. Urine and perfusate samples were stored at -20°C until analysis.

Respiration measurements

The effect of iohexol on cellular and mitochondrial respiration was determined in rat isolated kidney proximal tubular cells and kidney cortex mitochondria. Proximal tubular cells were isolated as described previously (Masereeuw *et al.*, 1994) and suspended to 10–15 mg protein ml^{-1} in incubation buffer containing (mM): NaCl 117.5, KCl 4, MgSO_4 1.2, KH_2PO_4 0.95, NaHCO_3 22.5, glucose 11.1 and CaCl_2 2.5. Rat kidney cortex mitochondria were isolated as described by Cain & Skilleter (1987), with some modifications. All steps were carried out at 4°C . Briefly, kidneys were isolated after perfusion with an ice-cold solution containing 140 mM NaCl and 10 mM KCl. The capsula was removed, medulla was dissected and cortex was collected in a Potter-Elvehjem homogenizer with Teflon pestle (clearance 0.5 mm) in three times the tissue weight of homogenization buffer (300 mM mannitol, 10 mM HEPES, 1 mM EGTA, 1 mg ml^{-1} BSA at pH 7.4). Tissue was homogenized gently six times by hand, and suspension was centrifuged for 10 min at 500 g. Supernatant was collected and centrifuged for 7 min at 11,000 g. Pellet was washed with homogenization buffer and again centrifuged (7 min, 11,000 g). Final pellet was diluted to a concentration of 5 mg ml^{-1} mitochondrial protein in respiration medium (210 mM mannitol, 10 mM KCl, 10 mM KH_2PO_4 , 0.5 mM EGTA, 60 mM Tris-HCl, at pH 7.4).

Respiration measurements were done with a Clarke-type platinum electrode, with 1 mg of cellular or mitochondrial protein in 2.0 ml of medium. Basal cellular O_2 consumption was measured at 37°C in incubation buffer supplemented with 4 mM sodium lactate, 1 mM alanine, and 10 mM butyric acid (pH 7.4). Uncoupled cellular respiration was determined in the presence of 44 μM dinitrophenol (DNP). Mitochondrial oxygen consumption was measured at 30°C in respiration medium in the absence of adenosine 5'-diphosphate (ADP) (state 2), in the presence of ADP (state 3), after ADP consumption (state 4), and after the addition of DNP, (final concentration 44 μM). Succinate (10 mM) was used as the metabolic substrate, and rotenone (1 μM) was added to block electron transport proximal to succinate entry into the respiratory chain. ADP-stimulated respiration (state 3) was measured in the presence of 0.3 mM ADP. Respiratory rates were calculated and expressed as nanogram atoms of oxygen per minute per milligram of protein (ng atom $\text{O min}^{-1} \text{mg}^{-1} \text{prot.}$).

Analysis

Urine and perfusate samples were analyzed for electrolytes and glucose, as described in a previous paper from our department (Cox *et al.*, 1990). The concentration of iohexol in perfusate and urine samples were determined by use of reversed-phase high-performance liquid chromatography (h.p.l.c.), as described below. This h.p.l.c. method could not be used for the determination of iohexol concentrations in kidney tissue, because iohexol could not be separated from all tissue components. Concentrations in the kidneys were determined by a chemical method according to Bäck *et al.* (1988). The kidneys were homogenized in 5 ml distilled water with a Polytron homogenizer on setting 10 for 2 times 60 s. A sample of 50 μl was used for further determination. Inulin concentration in kidney tissue and in perfusion samples were determined according to Heyrovski (1956). Protein content in each proximal tubular cell and kidney cortex mitochondrial preparation was determined by use of the Bio-Rad Protein Assay from Bio-Rad (München, Germany) with BSA as protein standard.

H.p.l.c. assay

A 1084B Liquid Chromatograph of Hewlett Packard (Böblingen, Germany) was used, equipped with an auto-injector (HP 79841 A), terminal (HP 79850 B LC) and an u.v. absorbance detector (Spectroflow 773, Kratos analytical instruments, Ramsey, N.J., U.S.A.) at an operating wavelength of 254 nm. Chromatography was performed on a stainless steel column (125 \times 4 mm) packed with LiChrospher 60 RP-Select B (Merck, Darmstadt, Germany), particle size 5 μm . The mobile phase consisted of 0.01 M potassium dihydrogen phosphate buffer (pH 2.6) and flow rate was 0.3 ml min^{-1} . Iodopyracet was used as internal standard (0.1 mg ml^{-1}). With a column temperature of 40°C , iohexol eluted as two peaks with retention times of 7.4 and 8.6 min with a ratio of 1:4. Within the same run, iodopyracet eluted with a retention time of 10.4 min. Sample preparation was performed with YM-10 ultrafiltration membranes (13 mm i.d.) with a molecular weight cut-off of 10,000 in the MPS-1 micropartition system (Amicon, Grace BV, Capelle a/d IJssel, The Netherlands) to separate iohexol and pluronic F-108. Onto the membrane, 20–30 μl urine or 50–150 μl perfusate sample was pipetted, together with 50 μl of internal standard. Total volume was adjusted to 300 μl with mobile phase buffer (pH 2.6). The filter units were centrifuged for 20 min at 3,000 g and 180 μl of the filtrate was mixed with 320 μl mobile phase buffer. An aliquot of 20 μl of the resulting solution was injected onto the column. Concentrations in perfusate and urine were determined by comparing the peak area ratio of the second (and major) peak of iohexol and internal standard with a calibration curve of peak area ratio vs. iohexol concentration spiked to blank perfusate and urine. Linear calibration curves were obtained in all cases ($r^2 > 0.98$). The interday precision of the h.p.l.c. assay was determined by measuring a spiked perfusate and urine sample with each run. The coefficient of variation was found to be 8.7% for the perfusate sample (10 $\mu\text{g ml}^{-1}$, $n=11$) and 10.7% for urine (100 $\mu\text{g ml}^{-1}$, $n=12$).

Renal excretion

The renal excretion of iohexol appeared to be composed of glomerular filtration and saturable absorption, which was assumed to take place at the luminal side. Since plasma proteins were not present in perfusate and iohexol did not bind to any of the perfusate constituents, drug concentrations can be considered as unbound. The renal excretion rate of iohexol can therefore be expressed as:

$$R_R = Q_{GF} \cdot C_P - \frac{V_{A, \text{Max}} \cdot K_A}{K_A + C_P} \quad (1)$$

Renal clearance is described by:

$$Cl_R = \frac{R_R}{C_P} \quad (2)$$

where Q_{GF} = glomerular filtration rate (ml min^{-1}); R_R = renal excretion rate ($\mu\text{g min}^{-1}$); Cl_R = renal clearance (ml min^{-1}); C_P = drug concentration in perfusate ($\mu\text{g ml}^{-1}$); $V_{A, \text{Max}}$ = maximum rate of absorption ($\mu\text{g min}^{-1}$); K_A = Michaelis-Menten constant of absorption ($\mu\text{g ml}^{-1}$).

Materials

Pluronic F-108 was from BASF (Arnhem, The Netherlands) and cyanocobalamin was obtained from Sigma (St. Louis, MO.). Iohexol was purchased from Nycomed (Oslo, Norway), diatrizoate, probenecid and poly-L-lysine (Mw 1,000–4,000) were from Sigma (St. Louis, MO.), iodopyracet was obtained from Dagra (Diemen, The Netherlands). Bovine serum albumin (BSA) and 4 - (2 - hydroxyethyl) - 1 - piperazineethanesulphonic acid (HEPES) were from Boehringer Mannheim (Mannheim, Germany). All other chemicals were of analytical grade and purchased from either Sigma (St. Louis, MO.) or Merck (Darmstadt, Germany).

Data analysis

All data are expressed as mean \pm s.d. Statistical differences between means were determined with Student's *t* test, in which the level of significance was set to $P < 0.05$. Renal excretion rate data were weighed reciprocally ($1/Y^2$), and analyzed according to equation 1 by the nonlinear least square regression program PCNonlin (Metzler & Weiner, 1986). The goodness of fit was evaluated through the deviation between observed and model predicted values as $R^2 = 1 - \Sigma(\text{Dev})^2 / \Sigma(\text{Obs})^2$, where $\Sigma(\text{Obs})^2$ is the observed sum of squared observations and $\Sigma(\text{Dev})^2$ is the sum of squared deviations.

Results

Kidney function

The mean values for the functional parameters of baseline period (–30–0 min) of all IPK experiments are presented in Table 1 ($n = 32$). After administration of iohexol, only small changes in renal function were observed. Compared to control IPK experiments, the renal perfusion pressure and fractional excretions of electrolytes and glucose were not altered. GFR, and urinary flow and pH were slightly changed in comparison to control experiments, and plotted against time after normalizing data to their control values (Figure 1a). At a perfusate concentration of $5 \mu\text{g ml}^{-1}$ iohexol, pretreatment with diatrizoate, probenecid or polylysine abolished the functional alterations (Figure 1b).

Renal excretion of iohexol

Iohexol was slowly eliminated from perfusion fluid. Figure 2 shows the time course of the mean perfusate concentration and corresponding excretion rate data of the four iohexol doses studied, as a function of time. All doses gave linear concentration-time curves. The urinary excretion rate increased rapidly after addition of iohexol, followed by a gradual decrease. Renal handling data are presented in Table 2. At a low perfusate concentration ($5 \mu\text{g ml}^{-1}$) the renal clearance was significantly lower than the clearance by glomerular filtration ($Cl_R/GF < 1$), indicating reabsorption of iohexol. At higher iohexol concentrations the ratio Cl_R/GF increased until 1, indicating that a saturable mechanism is involved in the luminal disappearance of the drug.

A plot of the renal excretion rate against perfusate concentration, a so called tubular titration curve, is presented in Figure 3. The dotted line represents clearance by glomerular

Table 1 Experimental conditions of the rat isolated perfused kidney (IPK) in the baseline period of all IPK experiments^a

Renal functional parameter	Value
FE _{glucose} (%)	3.1 ± 0.5
FE _{Na} (%)	1.8 ± 0.5
FE _K (%)	25 ± 8
FE _{Ca} (%)	2.4 ± 0.7
FE _{Mg} (%)	44 ± 12
GFR ($\mu\text{l min}^{-1}$)	414 ± 47
Urinary flow ($\mu\text{l min}^{-1}$)	21 ± 2
Urinary pH	5.5 ± 0.2
RPP (mmHg)	88 ± 5
FR _{water} (%)	94.9 ± 0.4
Perfusate flow (ml min^{-1})	12 ± 1

^aData shown are means \pm s.d., $n = 32$. FE = fractional excretion, FR = fractional reabsorption, RPP = renal perfusion pressure.

filtration only. The line through the data points was obtained after analyzing renal excretion data over the period 30 to 90 min, according to equation 1. The tubular titration curves for compounds that are actively reabsorbed, run below the glomerular filtration line and parallel it as this mechanism becomes saturated. Because the currently observed titration curve for iohexol diverges from the theoretical titration curve for active reabsorption, another approach had to be used to describe renal excretion data (equation 1). It is suggested that the drug binds to the luminal membrane of the kidney tubules and, subsequently, is internalized within the tubular cells. Saturation of this absorption mechanism appeared from the titration curve: at higher perfusate concentration the renal excretion rate line coincided with the glomerular filtration line. The insert of Figure 3 shows that this situation continues at perfusate concentrations up to $225 \mu\text{g ml}^{-1}$. Parameters obtained after fitting equation 1 to the data were a $V_{A, \text{Max}}$ of $1.4 \pm 0.1 \mu\text{g min}^{-1}$ and a K_A of $7.3 \pm 1.3 \mu\text{g ml}^{-1}$. The coefficient of determination (R^2) was 0.968.

To investigate further iohexol binding, studies were performed in which the perfused kidneys were pretreated with diatrizoate, probenecid, or polylysine. All three agents were able to enhance iohexol clearance at the lowest perfusate concentration ($5 \mu\text{g ml}^{-1}$) (Table 2). Figure 4 shows the ratio of renal clearance over clearance by glomerular filtration during the experimental period (0–120 min) for all treatments. The ratio increased during the first 30 min of the experiment until a maximum, and remained stable for the rest of the time. The plateau value of the Cl_R/GF ratio showed a dose-dependency and reached 1 at a perfusate concentration of $20 \mu\text{g ml}^{-1}$. It is obvious that all three compounds enhanced iohexol clearance over the whole experimental period.

Renal accumulation of iohexol

The concentration of iohexol in the kidney was expressed as the amount of drug per weight of tissue, and is presented in Table 2. The concentration in kidney tissue divided by the concentration in perfusate at the end of the experiment, resulted in an accumulated ratio (kidney/perfusate ratio). Iohexol accumulation in kidney tissue (kidney/perfusate ratio > 1), and it was observed that at a low perfusate concentration the accumulation ratio tended to be high and at high perfusate concentration it tended to be low, suggesting that a saturable mechanism is involved in drug tissue uptake. As a control, in three separate perfusion experiments the concentration of the filtration marker inulin was determined in kidney tissue and perfusion fluid, and a kidney/perfusate ratio of 0.123 ± 0.007 was found. This indicates that accumulation of iohexol is a result of specific tubular cell binding and uptake,

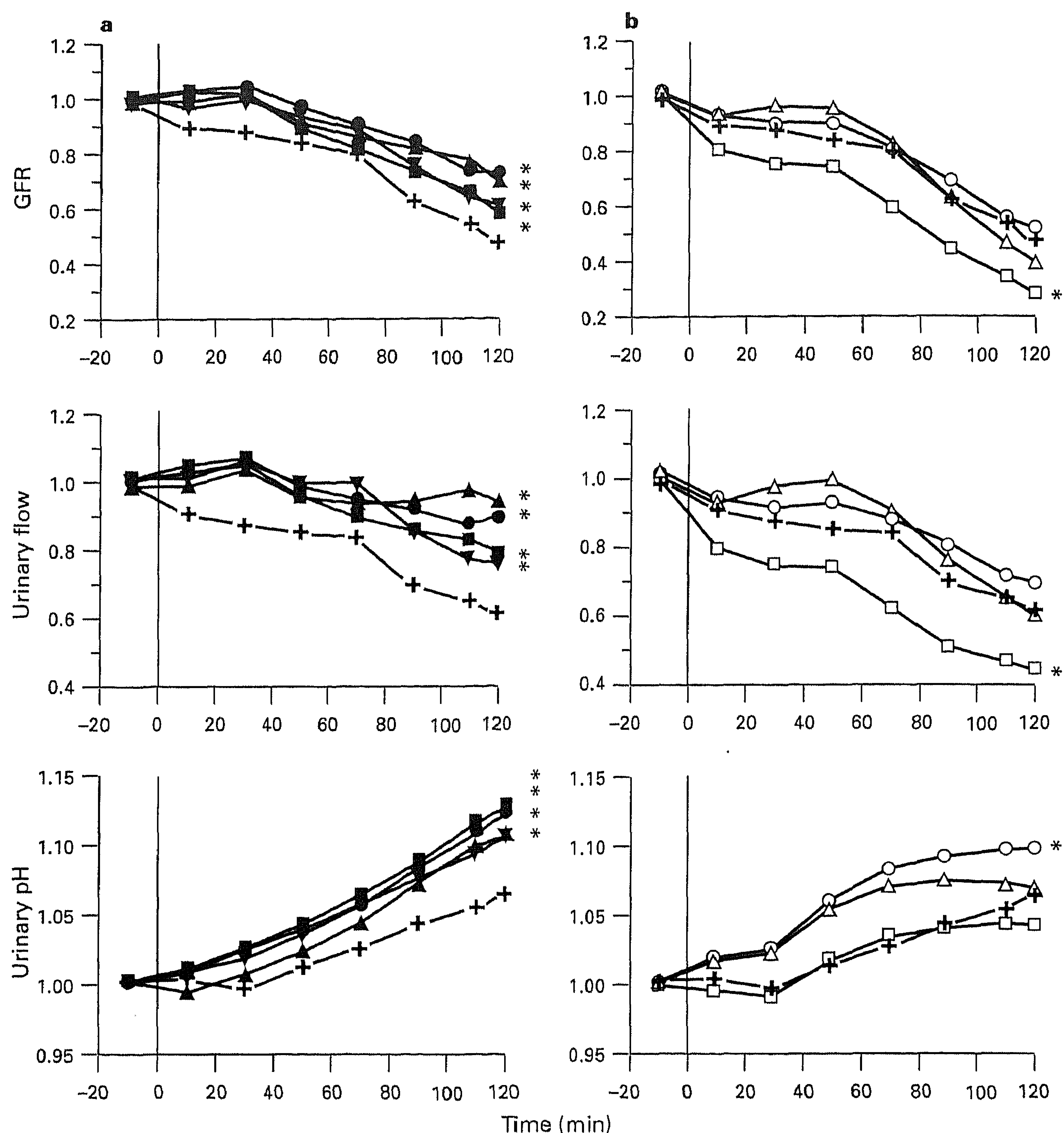


Figure 1 Glomerular filtration rate (GFR), urinary flow and urinary pH, as a function of time are shown. Renal functional values are normalized for the mean value of the baseline period (–30 to 0 min). Iohexol was administered at time $t=0$ min. Asterisks indicate significantly different from control ($P<0.05$), tested over the 30 to 120 min experimental period. (a) The effects on kidney function of 4 different perfusate concentrations of iohexol and the results from control IPK (isolated perfused kidney) experiments are presented ($n=4$). Concentrations used were: non (control, +); iohexol $5\mu\text{g ml}^{-1}$ (●); $7.5\mu\text{g ml}^{-1}$ (▲); $10\mu\text{g ml}^{-1}$ (■) and $20\mu\text{g ml}^{-1}$ (▼). (b) Kidney function in control IPK experiments (+, $n=4$) and at a perfusate concentration of $5\mu\text{g ml}^{-1}$ iohexol together with $16\mu\text{g ml}^{-1}$ polylysine (□, $n=5$), $200\mu\text{g ml}^{-1}$ diatrizoate (○, $n=3$), or $86\mu\text{g ml}^{-1}$ probenecid (△, $n=4$).

and that the contribution of concentration in extracellular fluid and tubular lumen to total tissue accumulation is negligible. Pretreatment with probenecid, diatrizoate and polylysine resulted in a diminished tissue accumulation of iohexol, although kidney/perfusate ratios were not significantly altered.

Respiration measurements

Effect of iohexol on cellular and mitochondrial oxygen consumption is illustrated in Figure 5. A concentration of $80\mu\text{g ml}^{-1}$ iohexol was used, which is comparable to the maximum concentration found in kidney tissue (Table 2). In proximal tubular cells, basal respiration was not affected but DNP-stimulated respiration was significantly inhibited by iohexol. In our mitochondrial preparation DNP-stimulated respiration was also significantly reduced, indicating a reduction in oxidative metabolism. Iohexol did not alter basal and ADP stimulated mitochondrial oxygen consumption. A respiratory control ratio (ratio of state 3 respiration over state 4 respiration) of 3.5 ± 0.1 was determined for the control situation, indicating that our mitochondrial preparation is of good quality and tightly coupled. A ratio of 3.4 ± 0.3 was calculated in the presence of iohexol.

Discussion

Clearance data of iohexol in the IPK showed that an absorptive mechanism is involved in its renal handling, which is assumed to be initiated by binding to the brush-border membrane. It has been speculated previously that contrast agent molecules might enter tubular cells from the lumen, because high iodine concentrations were found at the brush-border region shortly after administration (Nordby *et al.*, 1990). However, the presence of such an absorptive mechanism was never demonstrated. Our kinetic model, comprising glomerular filtration and saturable absorption, is able to describe the renal clearance data adequately. At perfusate concentrations above $20\mu\text{g ml}^{-1}$ saturation of absorption occurs. It is most likely that because of the high doses used in human studies, an absorptive mechanism was never observed, and iohexol was supposed to be suitable as a filtration marker. Most studies in human subjects were performed with doses between 3 and 7 g, resulting in initial plasma concentrations of approximately 1000 times higher than used in our IPK experiments (Frennby *et al.*, 1994; Lindblad & Berg, 1994). According to our results, at these concentrations renal clearance will indeed be determined only by glomerular filtration.

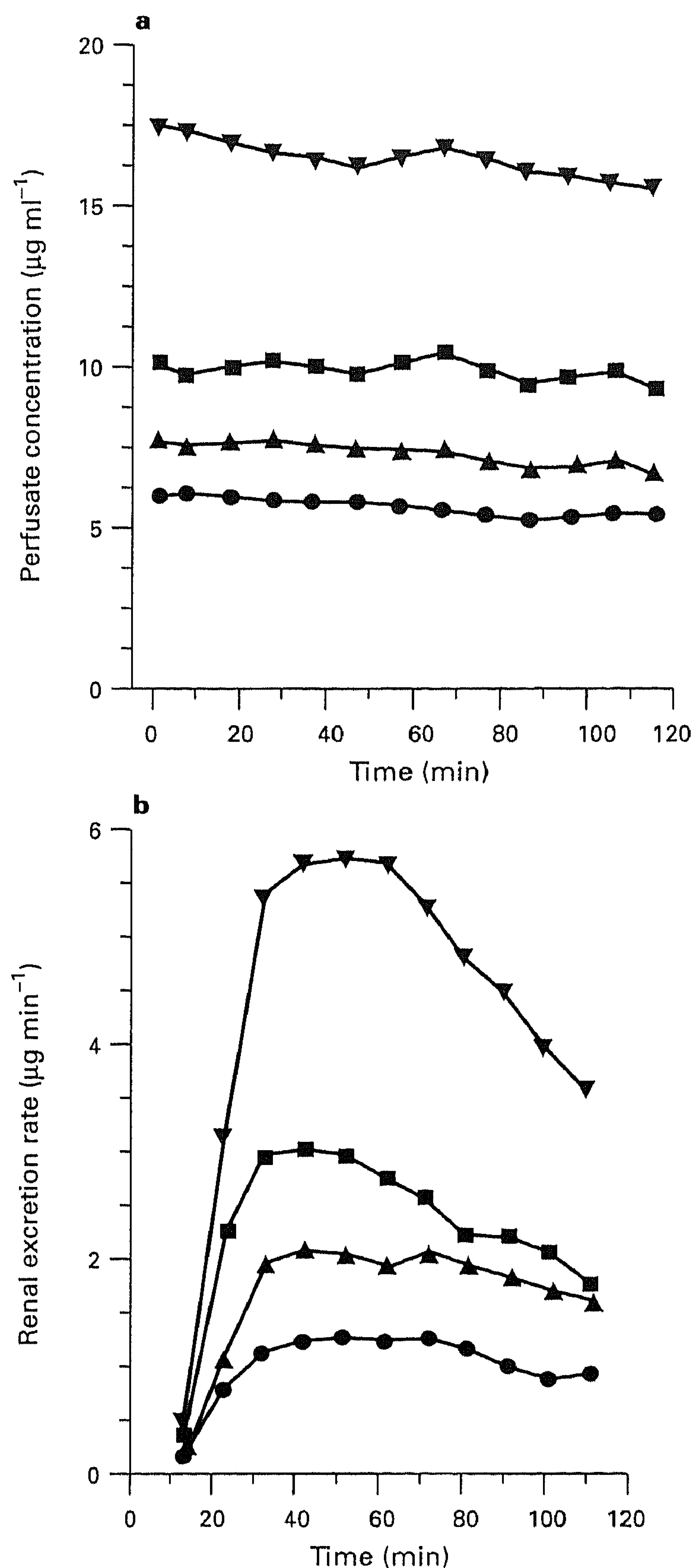


Figure 2 Iohexol perfusate concentration (a) and urinary excretion rate (b) as a function of time. Doses added to the perfused kidneys were 1.25 (●); 1.88 (▲); 2.5 (■) and 5.0 mg (▼). All data points are means of 4 experiments. For the sake of clarity standard deviations were omitted from this figure, but varied between 5 and 18% for perfusate concentration, and between 10 and 35% for renal excretion rate values.

The renal clearance of iohexol was enhanced after pretreatment with diatrizoate, polylysine, or probenecid, probably due to inhibition of membrane binding. Polylysine has been shown to inhibit aminoglycoside binding to brush-border membranes of rat kidney cortex. Moreover, *in vivo* nephrotoxicity could be prevented when coadministered with gentamicin (Williams *et al.*, 1986). It is possible that iohexol interacts with brush-border membrane components in a fashion similar to aminoglycosides. Subsequently, drug molecules are internalized in the tubular cells through an endocytotic mechanism, and sequestered within the lysosomal compartment. This sequestration may account for the observed vacuolization for iohexol in the proximal tubular cells (Tervahartiala *et al.*, 1991; Beaufils *et al.*, 1995). Although Heyman *et al.* (1988) previously suggested that vacuoles are not a result of endocytosis, but formed after membrane injury induced by contrast agents from the paracellular zones. The clearance profile and tissue accumulation of iohexol in our IPK preparations, however, indicate that a re-absorptive process for the drug exists. Although we cannot differentiate between basolateral or luminal binding in the IPK, it seems likely that the luminal membrane is involved. Additional binding studies with isolated membrane vesicles of both membranes are needed to discriminate between basolateral and luminal binding. Supporting evidence for the luminal binding is provided by various studies on disruption of the brush-border membrane by contrast media. Tubular cells seem to be even more sensitive to membrane injury than to lysosomal disturbance after contrast medium administration (Thomsen *et al.*, 1988; Bakris, 1993). This is comparable to the membrane damage caused by aminoglycoside antibiotics, and supports our hypothesis that iohexol is handled by the kidney in a way comparable to aminoglycoside antibiotics. It is assumed that the cationic aminoglycosides bind to anionic phospholipids within the brush-border membranes (Kaloyanides, 1992). Whether membrane binding of nonionic iohexol molecules is based on a similar charge interaction between the amino groups of this contrast agent and membrane phospholipids, demands further research.

The inhibitor of organic anion transport, probenecid, also affected the renal clearance of iohexol. The effect of probenecid was investigated, because the tubular titration curve for iohexol in the IPK diverged from the theoretical curve, and a secretory component may be involved in the renal clearance of iohexol. In addition, other investigators suggested previously that the reduced renal accumulation of the contrast analogue diatrizoate, determined after pretreatment with probenecid, was a result of inhibition of organic anion transport (Mudge *et al.*, 1971). Contrary to these findings, probenecid enhanced the clearance of iohexol in our IPK preparations. This effect, however, may be a result of inhibition of tissue metabolism rather than an interaction with the binding site for iohexol. Previous studies have shown that probenecid affects glucose uptake (Kippen *et al.*, 1979) and organic cation transport (Choi & Kim, 1992) also, by a reduction in oxidative metabolism.

Table 2 Renal handling data of iohexol in the rat isolated perfused kidney^a

Dose (mg)	Perfusate conc. (µg ml ⁻¹)	Renal excretion rate (µg min ⁻¹)	Cl _R /GF	Amount in kidney (µg g ⁻¹)	Kidney/perfusate ratio
1.25	5.6 ± 0.6	1.2 ± 0.3	0.63 ± 0.06	39 ± 25	7.5 ± 5.1
1.88	7.4 ± 0.4	2.0 ± 0.2	0.79 ± 0.05	35 ± 21	3.1 ± 1.7
2.5	10 ± 1.6	2.7 ± 0.5	0.85 ± 0.06	34 ± 7	2.2 ± 0.3‡
5.0	16 ± 0.5	5.3 ± 0.1	1.02 ± 0.06	64 ± 16	2.7 ± 0.9
1.25 ^b	5.3 ± 0.4	1.8 ± 0.2*	0.93 ± 0.03*	25 ± 5	5.2 ± 1.5
1.25 ^c	5.9 ± 0.8	1.5 ± 0.2	0.77 ± 0.04*	31 ± 7	5.6 ± 1.9
1.25 ^d	4.9 ± 0.5	1.2 ± 0.2	0.81 ± 0.03*	15 ± 8	3.4 ± 2.0

^a Data shown are means ± s.d. (n = 3–5) over the period of 30 to 90 min, except for the amount in kidney which was determined at the end of the experiment. Cl_R/GF: ratio of renal clearance over clearance by glomerular filtration. ^b In the presence of 200 µg ml⁻¹ diatrizoate, ^c 86 µg ml⁻¹ probenecid, ^d 16 µg ml⁻¹ polylysine. *P < 0.05 in comparison with same dose without treatment. ‡P < 0.05 in comparison with same ratio after a dose of 1.25 mg.

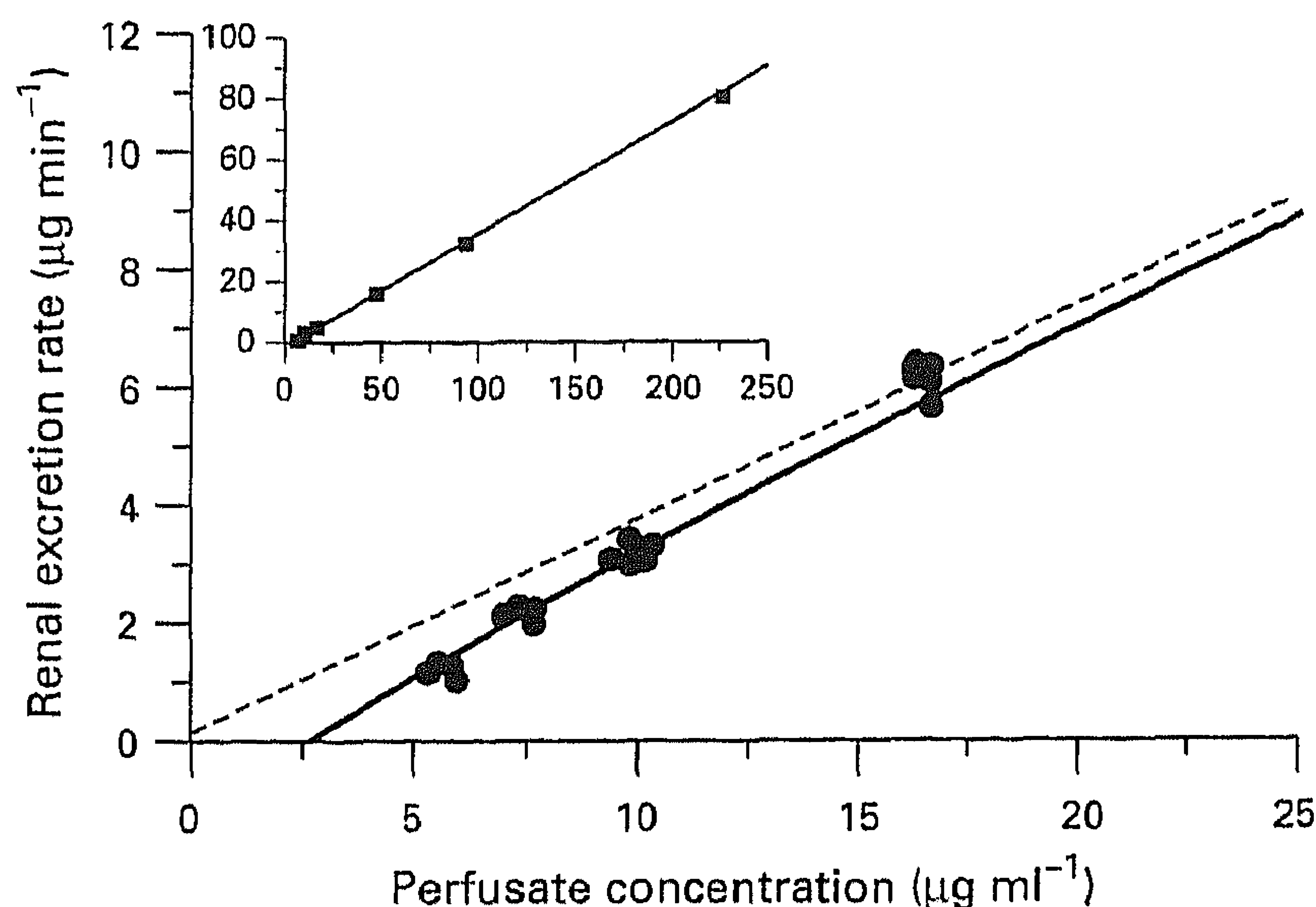


Figure 3 Tubular titration curve of iohexol. Renal excretion rate and rate of filtration as a function of perfusate concentration. Data obtained over the period 30–90 min are means of 4 experiments. The solid line represents the fit according to equation 1, the dashed line corresponds to the clearance by glomerular filtration only. All data were corrected for the fall in glomerular filtration rate (GFR) during the experiment. Insert: renal excretion rate as a function of perfusate concentration with 3 additional doses, resulting in initial perfusate concentrations 50, 100 and 250 $\mu\text{g ml}^{-1}$ ($n=1$). Solid and dashed lines are positioned on top of each other.

The effect of diatrizoate was investigated because this contrast analogue is structurally related to iohexol, but negatively charged. Renal stop-flow experiments in the dog showed that the renal clearance of diatrizoate over inulin clearance was 0.9 (Mudge *et al.*, 1971), indicating that a similar reabsorptive mechanism may be involved in diatrizoate clearance. The enhanced iohexol clearance after pretreatment with diatrizoate is probably a result of a decreased binding. Whether there is competition for the same binding site, remains to be elucidated.

At concentrations much lower than those used clinically, iohexol accumulated in kidney tissue reaching tissue concentrations of 2 to 7.5 times the medium concentration. We investigated whether internalized iohexol affected oxidative metabolism, as was found for high osmolar contrast agents (Humes *et al.*, 1987; Heyman *et al.*, 1988). Although a direct effect of iohexol on basal and ADP stimulated respiration was not observed, the uncoupled respiratory rate was significantly reduced in both cell and mitochondrial preparations. The uncoupling agent dinitrophenol discharges the proton electrochemical gradient across the mitochondrial inner membrane, thereby stimulating oxygen consumption without ATP synthesis. Reduction in uncoupled respiration is an indication of loss of respiratory control and a reduction in mitochondrial oxidative metabolism (Cain & Skilleter, 1987), indicating that this low osmolar contrast agent may induce direct tubular cell injury. Similar results were obtained with the high osmolar agent diatrizoate, and it was suggested that mitochondrial Na-K-ATPase was affected (Humes *et al.*, 1987; Porter, 1994). Heyman *et al.* (1988, 1991) observed mitochondrial swelling and vacuolization after iohalamate administration, mainly in the medullary thick ascending limb. However, they concluded that renal injury through contrast media was indirect and induced by a limited oxygen supply in that part of the nephron. Humes *et al.* (1987) showed that direct tubular cell injury may be additive to hypoxic cell injury in radiocontrast-induced renal dysfunction. Although our perfused kidney preparation exhibited signs of medullary thick ascending limb defects after 150 min of perfusion, and also hypoxia in proximal tubules may occur (Cox *et al.*, 1990), we did not expect this to have a major influence on our results, because in most experiments data over the period of 30–90 min were used. Furthermore, hypoxia limits all active transport processes by reduction of cellular energy, and therefore, reabsorption of iohexol may have even been underestimated as compared to the *in vivo* situation.

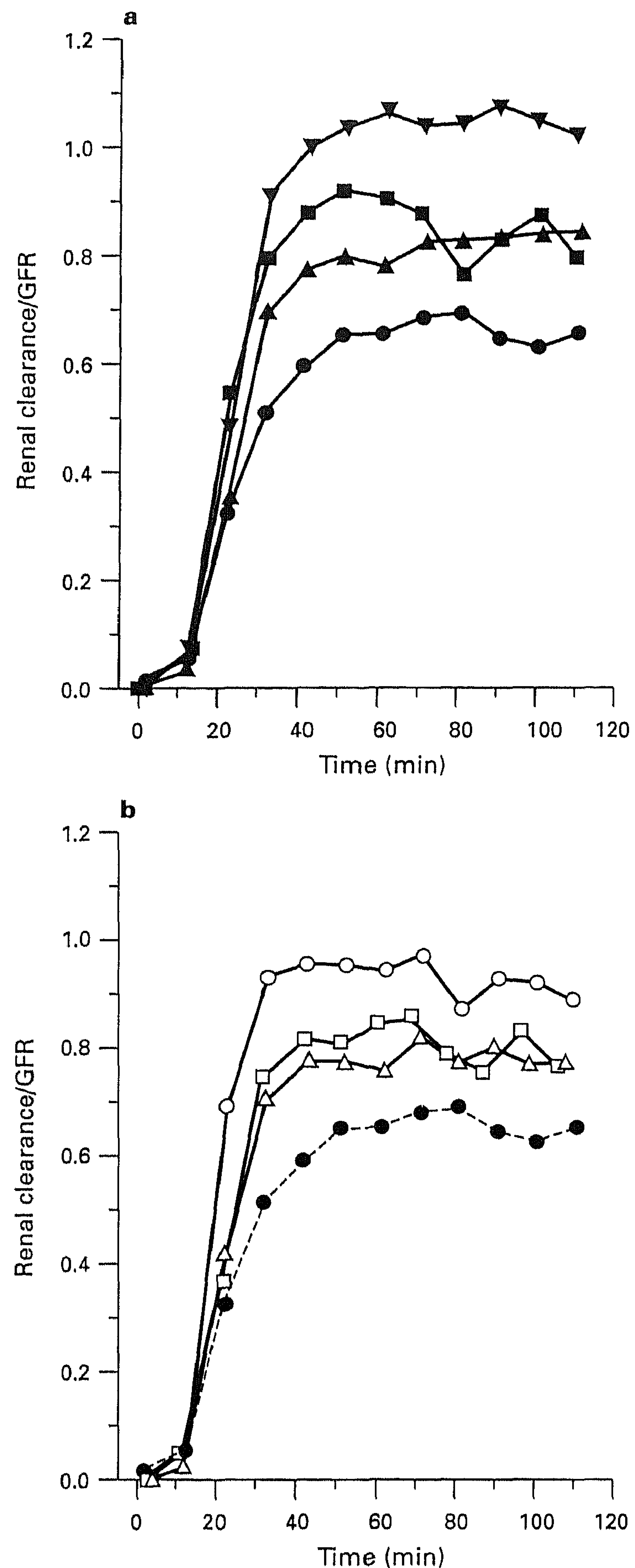


Figure 4 Renal clearance over glomerular filtration clearance (GFR) of iohexol as a function of time. (a) Four different perfusate concentrations of iohexol were studied: 5 $\mu\text{g ml}^{-1}$ (●), 7.5 $\mu\text{g ml}^{-1}$ (▲), 10 $\mu\text{g ml}^{-1}$ (■) and 20 $\mu\text{g ml}^{-1}$ (▼). (b) A concentration of 5 $\mu\text{g ml}^{-1}$ iohexol was used, after preincubation with diatrizoate (○, 200 $\mu\text{g ml}^{-1}$), probenecid (△, 86 $\mu\text{g ml}^{-1}$) or polylysine (□, 16 $\mu\text{g ml}^{-1}$). Data are means of 3 to 5 experiments. Standard deviations varied between 15 and 29%.

With regard to our results, the use of iohexol as a filtration marker may have clinical implications. In patients with impaired renal function, especially, the exposure time to contrast media is lengthened as a result of a prolonged half-life, and drug accumulation may be more extensive. Previous studies have shown that renal insufficiency is an important risk factor in the development of contrast-associated nephropathy (Barrett *et al.*, 1992; Porter, 1994).

In summary, renal handling of iohexol involves filtration and saturable absorption, resulting in tubular accumulation. The process of absorption is assumed to be initiated by binding of iohexol to the brush-border membrane, and can be inhibited

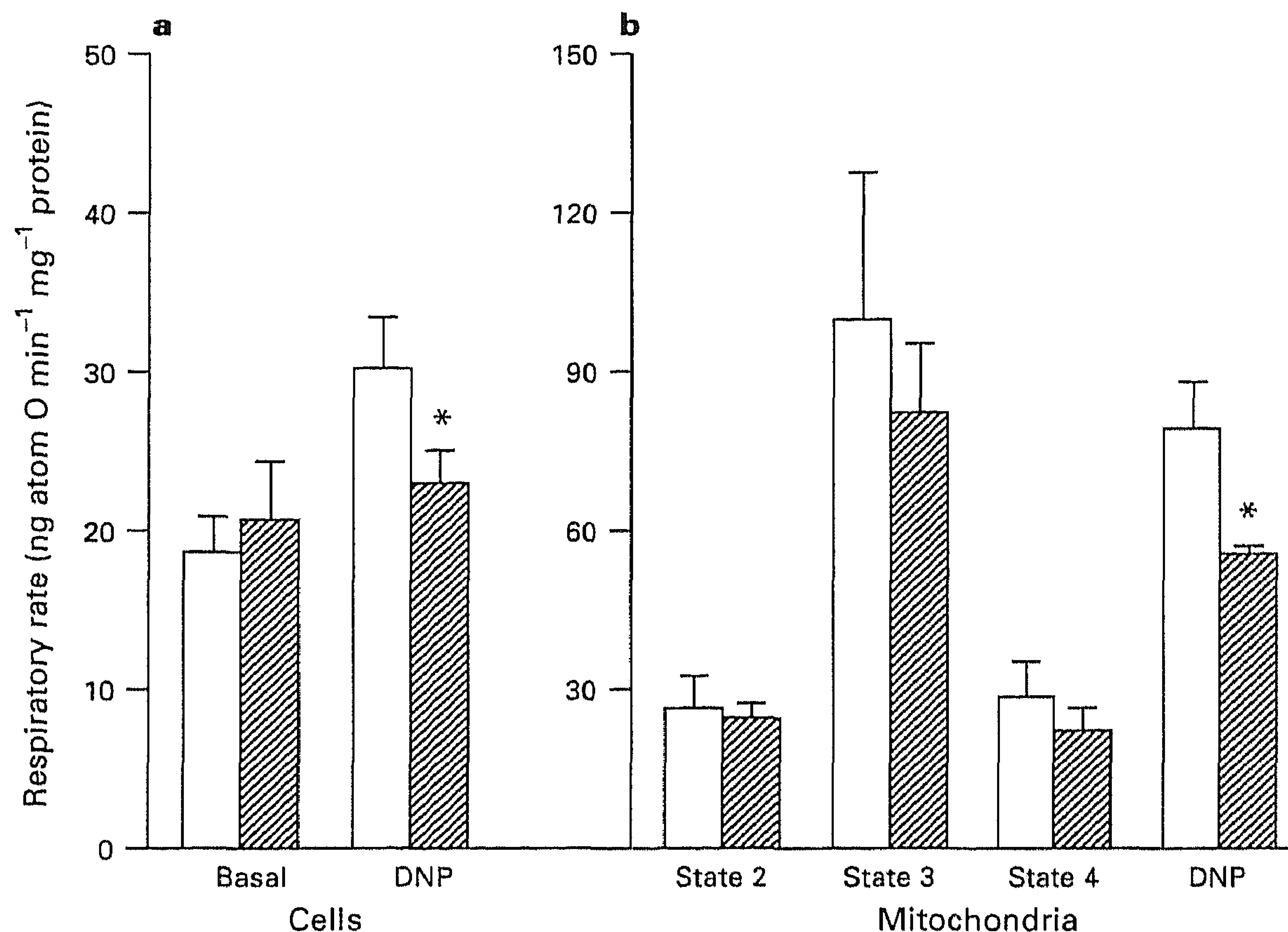


Figure 5 Respiratory rate of rat freshly isolated kidney proximal tubular cells (a) and mitochondria (b), in control situation (open columns) and in the presence of $80 \mu\text{g ml}^{-1}$ iohexol (hatched columns). State 2 respiration was measured before the addition of ADP, state 3 is the ADP-stimulated respiration, state 4 is the respiration measured after ADP consumption, and DNP is the dinitrophenol-stimulated respiration. Data are means \pm s.d. (vertical lines) of 4–7 different preparations, asterisks indicate significantly different from control ($P < 0.05$).

by polylysine, diatrizoate and probenecid. Intracellularly accumulated contrast medium affects mitochondrial oxidative metabolism. As a consequence, iohexol is not a true marker of GFR.

This study was supported by a grant from the Dutch Kidney Foundation (grant # C.90.1047). William C. Saleming is gratefully acknowledged for his help in the respiration experiments.

References

- BÄCK, S.E., MASSON, P. & NILSSON-EHLE, P. (1988). A simple chemical method for the quantification of the contrast agent iohexol, applicable to glomerular filtration rate measurements. *Scand. J. Clin. Lab. Invest.*, **48**, 825–829.
- BAKRIS, G.L. (1993). Pathogenesis and therapeutic aspects of radiocontrast-induced renal dysfunction. In *Toxicology of the Kidney* (2nd ed.), ed. Hook, J.B. & Goldstein, R.S. pp 361–386. New York: Raven Press, Ltd.
- BARRETT, B.J., PARFEY, P.S., VAVASOUR, H.M., McDONALD, J., KENT, G., HEFFERTON, D., O'DEA, F., STONE, E., REDDY, R. & MCMANAMON, P.J. (1992). Contrast nephropathy in patients with impaired renal function: high versus low osmolar media. *Kidney Int.*, **41**, 1274–1279.
- BEAUFILS, H., IDÉE, J.M., BERTHOMMIER, C., BALUT, C., BOURBOUZE, R., NIMIER, K., CHICANDRE-JOUANNEAU, C. & BONNEMAIN, B. (1995). Iobitridol, a new nonionic low-osmolality contrast agent, and iohexol. Impact on renal histology in the rat. *Invest. Radiol.*, **30**, 33–39.
- BOOM, S.P.A., MOONS, M.M. & RUSSEL, F.G.M. (1994). Renal tubular transport of cimetidine in the isolated perfused kidney of the rat. *Drug Metab. Dispos.*, **22**, 148–153.
- BRINK, H.M. & SLEGGERS, J.F.G. (1979). Instantaneous measurement of glomerular filtration in the isolated perfused rat kidney. *Pflügers Arch.*, **383**, 71–73.
- CAIN, K. & SKILLETER, D.N. (1987). Preparation and use of mitochondria in toxicological research. In *Biochemical Toxicology, A Practical Approach*, chapter 9, ed. Snell, K. & Mullock, B. pp. 217–254, Oxford: IRL Press.
- CHOI, T.L. & KIM, Y.K. (1992). Effect of probenecid on tetraethyl ammonium (TEA) transport across basolateral membrane of rabbit proximal tubule. *Kor. J. Int. Med.*, **7**, 130–136.
- COX, P.G.F., MOONS, M.M., SLEGGERS, J.F.G., RUSSEL, F.G.M. & VAN GINNEKEN, C.A.M. (1990). The isolated perfused rat kidney as a tool in the investigation of renal handling and effects of nonsteroidal anti-inflammatory drugs. *J. Pharmacol. Meth.*, **24**, 89–103.
- COX, P.G.F., MOONS, W.M., RUSSEL, F.G.M. & VAN GINNEKEN, C.A.M. (1991). Renal handling and effects of S(+)-ibuprofen and R(-)-ibuprofen in the rat isolated perfused kidney. *Br. J. Pharmacol.*, **103**, 1542–1546.
- FRENNBY, B., STERNER, G., ALMÉN, T., HAGSTAM, K.-E. & JACOBSSON, L. (1994). Determination of low glomerular filtration rate using iohexol clearance. *Invest. Radiol.*, **29**, S234–S235.
- HEYMAN, S.N., BREZIS, M., REUBINOFF, C.A.E., GREENFELD, Z., LECHENE, C., EPSTEIN, F.H. & ROSEN, S. (1988). Acute renal failure with selective medullary injury in the rat. *J. Clin. Invest.*, **82**, 401–412.
- HEYMAN, S.N., BREZIS, M., EPSTEIN, F.H., SPOKES, K., SILVA, P. & ROSEN, S. (1991). Early renal medullary hypoxic injury from radiocontrast and indomethacin. *Kidney Int.*, **40**, 632–642.
- HEYROVSKI, A. (1956). A new method for the determination of inulin in plasma and urine. *Clin. Chim. Acta*, **1**, 470–474.
- HUMES, H.D., HUNT, D.A. & WHITE, M.D. (1987). Direct toxic effect of the radiocontrast agent diatrizoate on renal proximal tubule cells. *Am. J. Physiol.*, **252**, F246–F255.
- KALOYANIDES, G.J. (1992). Drug-phospholipid interactions: role in aminoglycoside nephrotoxicity. *Renal Failure*, **14**, 351–357.
- KIPPEN, I., HIRAYAMA, B., KLINENBERG, J.R. & WRIGHT, E.M. (1979). Transport of p-animohippuric acid, uric acid and glucose in highly purified rabbit renal brush border membranes. *Biochim. Biophys. Acta*, **556**, 161–174.
- LINDBLAD, H.G. & BERG, U.B. (1994). Comparative evaluation of iohexol and inulin clearance for glomerular filtration rate determinations. *Acta Paediatr.*, **83**, 418–422.
- MASEREEUW, R., VAN DEN BERGH, E.J., BINDELS, R.J.M. & RUSSEL, F.G.M. (1994). Characterization of fluorescein transport in isolated proximal tubular cells of the rat: evidence for mitochondrial accumulation. *J. Pharmacol. Exp. Ther.*, **269**, 1261–1267.

- METZLER, C.M. & WEINER, D.L. (1986). Statistical Consultants, Inc. PCNONLIN and NONLIN84: Software for the statistical analysis of nonlinear models. *Am. Statist.*, **40**, 52.
- MORRIS, T.W. & FISCHER, H.W. (1986). The pharmacology of intravascular radiocontrast media. *Ann. Rev. Pharmacol. Toxicol.*, **26**, 143–160.
- MUDGE, G.H., BERNDT, W.O., SAUNDERS, A. & BEATTIE, B. (1971). Renal transport of diatrizoate in the rabbit, dog, and rat. *Nephron*, **8**, 156–172.
- NORDBY, A., TVEDT, K.E., HALGUNSET, J. & HAUGEN, O.A. (1990). Intracellular penetration and accumulation of radiographic contrast media in the rat kidney. *Scanning Microsc.*, **4**, 651–666.
- PORTER, G.A. (1994). Contrast-associated nephropathy: presentation, pathophysiology and management. *Miner. Electrolyte Metab.*, **20**, 232–243.
- SOVAK, M. (1994). Contrast media: a journey almost sentimental. *Invest. Radiol.*, **29**, S4–S14.
- TERVAHARTIALA, P., KIVISAARI, L., KIVISAARI, R., VIRTANEN, I. & STANDERTSKJÖLD-NORDENSTAM, C.-G. (1991). Contrast media-induced renal tubular vacuolization. A light and electron microscopic study on rat kidneys. *Invest. Radiol.*, **26**, 882–887.
- THOMSEN, H.S., DORPH, S., MYGIND, T., SOVAK, M., NIELSEN, H., RYGAARD, H., LARSEN, S., SKAARUP, P., HEMMINGSEN, L. & HOLM, J. (1988). Intravenous injections of ioxilan, iohexol and diatrizoate. Effects on urine profiles in the rat. *Acta Radiol.*, **29**, 131–136.
- WILLIAMS, P.D., HOTTENDORF, G.H. & BENNETT, D.B. (1986). Inhibition of renal membrane binding and nephrotoxicity of aminoglycosides. *J. Pharmacol. Exp. Ther.*, **237**, 919–925.

(Received December 18, 1995)

Revised May 7, 1996

Accepted May 29, 1996)